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N-terminal Isoforms of the Large-conductance Ca^{2+}-activated K^+ Channel Are Differentially Modulated by the Auxiliary β1-Subunit*

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Background: The large-conductance Ca^{2+}-activated K^+ channel (BK_{Ca}) is an important regulator of membrane excitability.
Results: N-terminal isoforms of the BK_{Ca} channel are differentially modulated by the regulatory β1-subunit.
Conclusion: Expression of different N-terminal isoforms is a novel mechanism of BK_{Ca} channel regulation.
Significance: Elucidating the modulation of BK_{Ca} activity by β1 provides a new understanding of ion channel physiology.

The large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel is essential for maintaining the membrane in a hyperpolarized state, thereby regulating neuronal excitability, smooth muscle contraction, and secretion. The BK_{Ca} α-subunit has three predicted initiation codons that generate proteins with N-terminal ends starting with the amino acid sequences MANG, MSSN, or MDAL. Because the N-terminal region and first transmembrane domain of the α-subunit are required for modulation by auxiliary β1-subunits, we examined whether β1 differentially modulates the N-terminal BK_{Ca} α-subunit isoforms. In the absence of β1, all isoforms had similar single-channel conductances and voltage-dependent activation. However, whereas β1 did not modulate the voltage-activation curve of MSSN, β1 induced a significant leftward shift of the voltage activation curves of both the MDAL and MANG isoforms. These shifts, of which the MDAL was larger, occurred at both 10 μM and 100 μM Ca^{2+}. The β1-subunit increased the open dwell times of all three isoforms and decreased the closed dwell times of MANG and MDAL but increased the closed dwell times of MSSN. The distinct modulation of voltage activation by the β1-subunit may be due to the differential effect of β1 on burst duration and interburst intervals observed among these isoforms. Additionally, we observed that the related β2-subunit induced comparable leftward shifts in the voltage-activation curves of all three isoforms, indicating that the differential modulation of these isoforms was specific to β1. These findings suggest that the relative expression of the N-terminal isoforms can fine-tune BK_{Ca} channel activity in cells, highlighting a novel mechanism of BK_{Ca} channel regulation.

The large-conductance voltage- and Ca^{2+}-activated K^+ channel (BK_{Ca})[2] is a key regulator of membrane excitability in a wide variety of cells such as neurons, smooth muscle, chromaffin cells, and immune cells (1–5). BK_{Ca} channel activation integrates both membrane depolarization and increases in intracellular Ca^{2+} to control membrane excitability (6). Although a single gene (KCNMA1) encodes the BK_{Ca} channel α-subunit, alternative splicing produces an array of BK_{Ca} isoforms that respond to a variety of modulators in tissue-specific manners (4, 7–9). Despite the high degree of splicing, all BK_{Ca} α-subunits are composed of seven conserved transmembrane domains (S0 through S6) and an extracellular N terminus. The N terminus and first transmembrane domain (S0) of the α-subunit are required for association with the auxiliary β1-subunit (10, 11), which is an important source of variable regulation for BK_{Ca} channel function (12–14).

Three possible translation initiation sites have been identified in the BK_{Ca} α-subunit N-terminal sequence (15, 16). Heterologous expression studies have used the third initiation site (methionine at position 66), starting with amino acid sequence MDAL, as the canonical human BK_{Ca} channel α-subunit to investigate β-subunit modulation (10, 14, 17). Other studies of the mouse (13, 18) or rat (9) BK_{Ca} channel have also used N-terminal truncated isoforms that start at the second or third translation initiation sites (starting with MSSN or MDAL, respectively). The full-length transcript, which can encode an isoform that starts with MANG, has been isolated from human smooth muscle (15), but whether the β1-subunit modulates this isoform of the human BK_{Ca} α-subunit has not been extensively investigated. Because the N-terminal region and first transmembrane domain (S0) are necessary for β1-subunit modulation (10, 11), we hypothesized that these three N-terminal isoforms of the BK_{Ca} α-subunit may be differentially modulated by the accessory β1-subunit. Here, we report that the β1-subunit differentially modulates the activation of the MANG, MSSN, and MDAL isoforms. We show that β1 modulates the voltage

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[2] The abbreviation used is: BK_{Ca}, large-conductance voltage- and Ca^{2+}-activated K^+ channel.

activation of the N-terminally extended isoforms less than that of the MDAL isoform. This effect may reflect differential modulation of channel kinetics of the three isoforms by β1. By contrast, the β2-subunit modulated voltage activation of MDAL, MSSN, and MANG isoforms to similar extents. These results suggest a new mechanism of fine-tuning the BK_{Ca} channel activity that depends on the expression of the N-terminal isoforms and their modulation by the β1-subunit.

EXPERIMENTAL PROCEDURES

Tissue Collection and Cloning of BK_{Ca} Isoforms—Non-pregnant human uteri were obtained from the Cooperative Human Tissue Network (Midwestern Division, Columbus, OH). Total RNA from human myometrium was reverse transcribed by using the First-strand RT-PCR kit (Stratagene, La Jolla, CA). The MANG (GenBank™ accession no. BC137137.1) and MSSN isoforms of the BK_{Ca} α-subunit were isolated from human myometrium by using sense primers 5′-ATGGCAAATGGTGGCGGC-3′ and 5′-ATGAGTAGCAATATCCAC-3′, respectively, with the antisense primer 5′-CCCAGTAGAGTCGTACTT-3′. The MDAL isoform (GenBank™ accession no. U11058.2) was subcloned by PCR using the MANG isoform as a template.

cDNA Constructs—All BK_{Ca} α-subunit N-terminal isoforms were inserted into the pCMV site of a pBudCE4.1 plasmid vector (Invitrogen) containing an optimal Kozak sequence (GACCACC) upstream of the start codon and including the mCherry reporter in the EF1-α site. The cDNA encoding the human β1-subunit (GenBank™ accession no. U25138.1) was cloned into the EF1-α site of pBudCE4.1; eGFP was cloned by PCR into the pCMV site as a reporter. The β2-subunit construct with its N terminus deleted (Δ2–20, β2ND) (19) was kindly provided by Dr. Jianmin Cui (Washington University in St. Louis) and was cloned into the EF1-α site of pBudCE4.1; eGFP was used as a reporter. Plasmid DNAs for transfection were isolated with a Plasmid Maxi kit (Qiagen, Hilden, Germany).

Cell Culture and Transfection—Human embryonic kidney (HEK) 293T cells were grown to 60–80% confluency in DMEM/F12 supplemented with 10% fetal bovine serum and 50 μg/ml gentamicin (all from Invitrogen). Cells were transiently transfected with constructs expressing the human BK_{Ca} channel N-terminal isoforms. Another set of cells was co-transfected with the BK_{Ca} α-subunit isoforms and the β1-subunit (in a 1:1 or 1:10 molar ratio) or the β2ND-subunit. All transfections were performed using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions. Cells were used in subsequent experiments 24–48 h post-transfection.

Electrophysiology—Single-channel recordings in the inside-out configuration were performed at room temperature in a bath solution containing the following: 140 mM KCl, 20 mM KOH, 10 mM HEPES, 5 mM (H)EDTA, and 10 μM or 100 μM free-Ca^{2+} (pH 7.2 with HCl). Free Ca^{2+} concentration was measured by using a Ca^{2+}-sensitive electrode (Thermo Fisher Scientific, Waltham, MA). Pipette solution contained the following: 140 mM KCl, 20 mM KOH, 2 mM $MgCl_2$, and 10 mM HEPES (pH 7.4). Single-channel currents were recorded at a sampling rate of 100 kHz and filtered at 5 kHz by using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). Currents were evoked with 10 mV voltage steps (1000-ms duration) from −160 to +120 mV, from a holding potential of 0 mV by using pCLAMP software (version 10, Molecular Devices). This protocol was repeated at least three times on each patch with 10 μM or 100 μM Ca^{2+} in the bath. For analysis purposes, recordings on the same patch were concatenated, attaining at least a 3-s length recording from each voltage pulse. Mean open probability (P_o) and unitary current (i) values were calculated by using pCLAMP software. Patches containing three or fewer channels were used for P_o analysis; only patches containing one channel were used for i measurements. P_o was plotted against membrane potential (V) and fitted to a Boltzmann function,

$$P_o = P_{o(max)}/(1+e^{-zF(V-V_{0.5})/RT}) \quad \text{(Eq. 1)}$$

to determine half-maximal activation voltage ($V_{0.5}$) and effective charge (z) values for each experiment by using Graph Pad software (San Diego, CA). Unitary conductance (γ) values were obtained from plotting the i-V relation (from −60 to +60 mV) and fitting to a linear regression; the slope of that regression was calculated as γ for each experiment. For analysis of open and closed dwell times of the channel (MANG, MSSN, or MDAL) in the presence or absence of β1-subunit, inside-out patches containing only one channel were held at −20 mV for 3 min with 10 μM Ca^{2+} in the bath. Open and closed dwell times histograms were plotted in log-bin time scales and fitted with double exponential functions to obtain time constants (τ) and relative distribution (P) of the data under the curve by using pCLAMP software. Burst analyses were performed on the same recordings as the dwell time analyses; bursts correspond to openings separated by a minimal closed interval defined as critical closed τ (τ_{crit}). τ_{crit} was estimated as the second or third component of a five component exponential function fitting within the closed dwell time histogram, as described previously (20). To ensure a clear estimation of τ_{crit}, only recordings with a P_o value less than 0.8 were included in the burst analysis.

Statistical Analysis—Data obtained were subjected to non-parametric Mann-Whitney U test by using Graph Pad software. A p value < 0.05 was considered significant. All data are presented as mean ± S.E.

RESULTS

BK_{Ca} N-terminal Isoforms Have Distinct Modes of Regulation by the Auxiliary β1-subunit—Previous studies identified three possible translation initiation sites in the BK_{Ca} α-subunit N-terminal sequence (15, 16). We cloned the longer N-terminal BK_{Ca} isoform, with amino acid sequence starting at MANG and the isoform starting at MSSN from human myometrium samples. Then, we used PCR to subclone the shorter N-terminal form starting at MDAL (Fig. 1); all constructs contained an optimal Kozak sequence at the 5′ end to optimize expression.

To test the functional properties of the N-terminal isoforms of the BK_{Ca} α-subunit, we expressed each construct individually in HEK293T cells and evaluated their voltage- and Ca^{2+}-dependent currents. Inside-out patch-clamp recordings showed a similar voltage- and Ca^{2+}-dependence of MANG, MSSN, and MDAL isoforms; all three channels showed a volt-

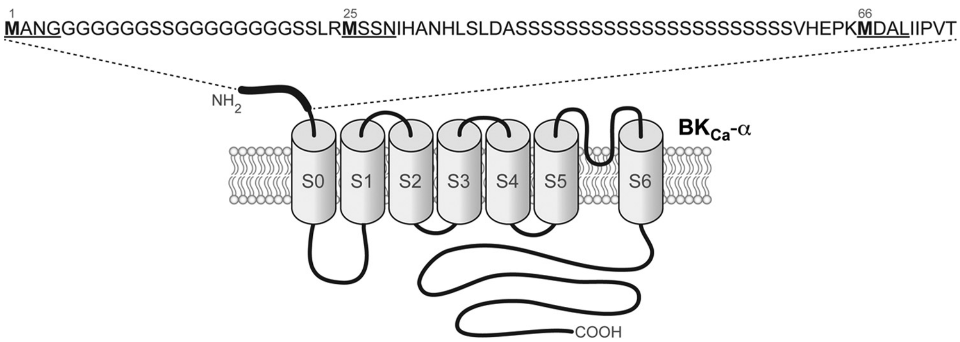

FIGURE 1. **BK_{Ca} channel α-subunit structure and N-terminal sequence.** A schematic representation of the human BK_{Ca} α-subunit is shown. The N-terminal peptide sequence of the α-subunit is shown in detail. Letters in *boldface type* show putative initiation methionines (Met-1, Met-25, and Met-66). *Underlined* stretches of four residues are names used in this study for each isoform. S0–S6 denote transmembrane domains.

age-induced increase in P_o (Fig. 2). Changes in Ca^{2+} concentration in the bath from 10 μM to 100 μM induced similar leftward shifts in the voltage-activation curves of MANG, MSSN, and MDAL (~50 mV, ~44 mV, and ~51 mV, respectively, Fig. 2 and Table 1). In addition to P_o, unitary conductances were comparable between these isoforms (Fig. 2*I*). Co-expression of the β1-subunit elicited a significant leftward shift in the voltage-activation curve of MDAL (~47 and ~53 mV at 10 and 100 μM Ca^{2+}, respectively, Fig. 2 and Table 1). Although the β1-subunit also shifted voltage-activation curves of the MANG isoform to the left, the shifts were significantly smaller than those of MDAL (~24 and ~25 mV at 10 and 100 μM Ca^{2+}, respectively, Fig. 2 and Table 1). In contrast, co-expression of β1 did not significantly shift the voltage-activation of the MSSN isoform (Fig. 2 and Table 1). The β1-subunit reduced unitary conductance only in the MDAL isoform (Fig. 2*I*). Moreover, the β1-subunit did not change the effective charge (*z*, given by the slope of voltage-activation curves) of any isoform at 10 μM or 100 μM Ca^{2+} (Table 1).

We further analyzed the effect of the β1-subunit on the MANG, MSSN, and MDAL channel kinetics by recording single channel currents at −20 mV for 3 min with 10 μM Ca^{2+} in the bath. In the presence of the β1-subunit, the open time constants (τ) were increased in MANG and MDAL, whereas only the larger τ (τ_2) was increased in MSSN (Fig. 3 and Table 2). Additionally, β1 decreased the τ of the closed state of both MANG and MDAL isoforms, but increased the τ of the closed state on MSSN (Fig. 3 and Table 2). Co-expression of β1 significantly increased the burst duration of both MDAL (from 18.0 ± 6.0 ms to 59.3 ± 18.5 ms, $p < 0.05$, Fig. 3*D*) and MSSN (from 23.9 ± 3.6 ms to 52.6 ± 10.2, $p < 0.05$, Fig. 3*D*), but not of MANG (from 11.5 ± 2.8 ms to 17.1 ± 6.9 ms, Fig. 3*D*). Interestingly, the interburst interval was increased on MANG and MSSN isoforms in the presence of the β1 subunit, but not in MDAL (Fig. 3*E*), indicating that the β1-induced stabilization of the burst state was different between these isoforms (Fig. 3*F*). Together, these observations suggest that the extended N-terminal sequence in the MANG isoform reduces, and in the case of MSSN abolishes, the β1-induced changes in voltage dependence of channel activation, which might be due, in part, to a differential β1-dependent modulation of channel kinetics.

A typical BK_{Ca} channel is thought to have a stoichiometry of 4α:4β1-subunits (21). However, the relative expression of each of these proteins has been reported to influence the overall effect of the β1-subunit on BK_{Ca} currents; binding of each β1 molecule can sequentially shift the $V_{0.5}$ of the channel (22). Therefore, it is feasible that the intermediate effect of β1 observed in the MANG isoform was due to fewer β1-subunit molecules binding to the α-subunit when expressed in 1:1 ratio. To address this possibility, we used an α:β1 cDNA ratio of 1:10 to express the MANG and MDAL isoforms in the presence of saturating concentrations of β1. The resulting voltage activation curves for both MANG and MDAL were similar to those observed when a 1:1 cDNA ratio was used (Fig. 4). Thus, the reduced β1-induced leftward shift of MANG voltage-activation curves was not due to fewer β1-subunits binding to this isoform.

All Three N-terminal Isoforms Are Similarly Modulated by the β2-subunit—Similar to β1, the related β2-subunit increases BK_{Ca} channel Ca^{2+} sensitivity (23), although it is likely that modulation by these subunits occurs by different mechanisms (19, 24, 25). To assess whether the significantly attenuated β1-induced leftward shift of the voltage-activation curve observed in the MANG isoform, and the lack of effect on the MSSN isoform, were specific for the β1-subunit, we tested the modulation of MANG, MSSN, and MDAL by β2. Because β2 inactivates the BK_{Ca} α-subunit channel currents by N-type inactivation, we used a truncated form of the β2-subunit (β2ND) that does not inactivate the channel (19, 23). We observed a significant leftward shift in the voltage-activation curves at two Ca^{2+} concentrations in cells co-expressing any of the α-subunit isoforms with β2ND as compared with those expressing α-subunit alone (curves were shifted to the left by ~50 mV and ~66 mV for MANG, by ~23 mV and ~58 mV for MSSN, and by ~59 mV and ~57 mV for MDAL, in the presence of 10 and 100 μM Ca^{2+}, respectively, Fig. 5). Hence, the decreased shift in channel activation by the extended N terminus of the MANG and MSSN isoforms is specific for the β1-subunit.

DISCUSSION

The BK_{Ca} channel plays an important role in regulating the membrane potential of excitable cells. Association of the pore-forming α-subunit of the BK_{Ca} channel with distinct auxiliary subunits (β1–β4 or the recently described γ1–γ4) is a significant form of channel modulation (17, 26). Accordingly, interaction of the α-subunit with the β1-subunit, which predomi-

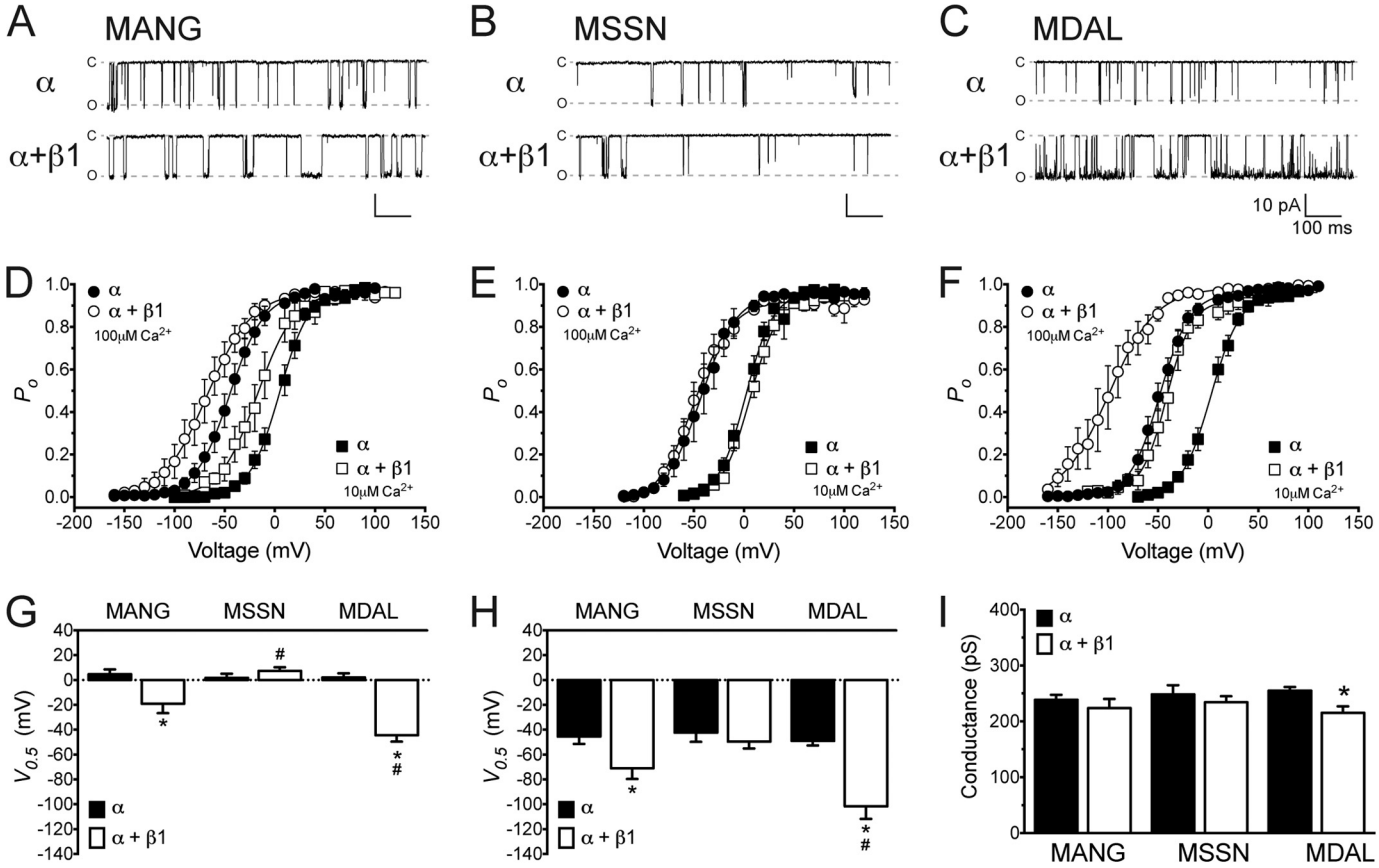

FIGURE 2. **MANG, MSSN, and MDAL isoforms of the BK_{Ca} channel are differentially modulated by the β1-subunit.** Representative excised patch recordings from HEK293T cells transfected with constructs expressing either the MANG (*A*), MSSN (*B*), or MDAL (*C*) α-subunit without (*upper traces*) or with (*lower traces*) β1-subunit, at −100 mV with 100 μM Ca^{2+} in the bath. *Dashed lines* indicate open (*O*) or closed (*C*) states of the channels. Voltage dependence of BK_{Ca} activation of MANG (*D*), MSSN (*E*), or MDAL (*F*) with (*open symbols*) or without (*closed symbols*) β1-subunit, expressed as open probability of the channel (P_o), in the presence of 10 μM (*squares*) or 100 μM (*circles*) Ca^{2+} ($n = 6–14$). Half-maximal activation voltage ($V_{0.5}$) obtained from voltage-activation curves for MANG, MSSN, or MDAL isoforms in 10 μM (*G*) or 100 μM Ca^{2+} (*H*) with (*white bars*) or without (*black bars*) β1-subunit. Single channel conductances of the different N-terminal isoforms (*I*) in the absence (*black bars*) or presence (*white bars*) of β1-subunit obtained at 10 μM Ca^{2+} ($n = 5–10$). *, $p < 0.05$ compared with α-subunit alone; #, $p < 0.05$ compared with MANG+β1 and MSSN+β1.

TABLE 1

Effect of β1-subunit on the voltage activation of different BK_{Ca} channel α-subunit N-terminal isoforms

	Half-maximal activation voltage ($V_{0.5}$) mV, mean ± S.E. (*n*)		Effective charge (*z*) mean ± S.E.	
Isoform	**α**	**α + β1**	**α**	**α + β1**
MANG				
10 μM Ca^{2+}	4.7 ± 3.9 (10)	−19.1 ± 7.6 (7)[a]	−2.26 ± 0.15	−2.15 ± 0.40
100 μM Ca^{2+}	−45.6 ± 5.9 (10)	−71.0 ± 8.7 (11)[a]	−2.00 ± 0.12	−1.84 ± 0.14
MSSN				
10 μM Ca^{2+}	1.7 ± 3.4 (10)	7.3 ± 3.0 (8)[b]	−2.46 ± 0.17	−2.87 ± 0.93
100 μM Ca^{2+}	−42.4 ± 7.3 (6)	−49.6 ± 5.6 (6)	−1.94 ± 0.12	−1.62 ± 0.26
MDAL				
10 μM Ca^{2+}	2.1 ± 3.3 (14)	−44.5 ± 5.2 (7)[c,d,e]	−2.36 ± 0.29	−3.14 ± 0.81
100 μM Ca^{2+}	−49.0 ± 3.6 (9)	−101.8 ± 10.0 (6)[b,c,e]	−2.09 ± 0.15	−1.73 ± 0.18

[a] $p < 0.05$ compared with α-subunit.
[b] $p < 0.05$ compared with MANG.
[c] $p < 0.01$ compared to α-subunit.
[d] $p < 0.01$ compared to MANG.
[e] $p < 0.01$ compared to MSSN.

nates in smooth muscle, confers increased Ca^{2+} sensitivity and decreased voltage-dependence to the BK_{Ca} channel (12, 13). Both the N-terminal region and the S0 transmembrane domain of the BK_{Ca} α-subunit are essential for both its interaction with the auxiliary β1-subunit (10, 11) and for β2-subunit modulation (19). In this work, we studied the properties of three different N-terminal isoforms (MANG, MSSN, and MDAL) and how they are modulated by the auxiliary β1- and β2-subunits. We observed that whereas the voltage-activation curve of MDAL was shifted to the left by ~50 mV, that of MANG was only shifted by ~25 mV, and that of the MSSN isoform was not affected by the β1-subunit.

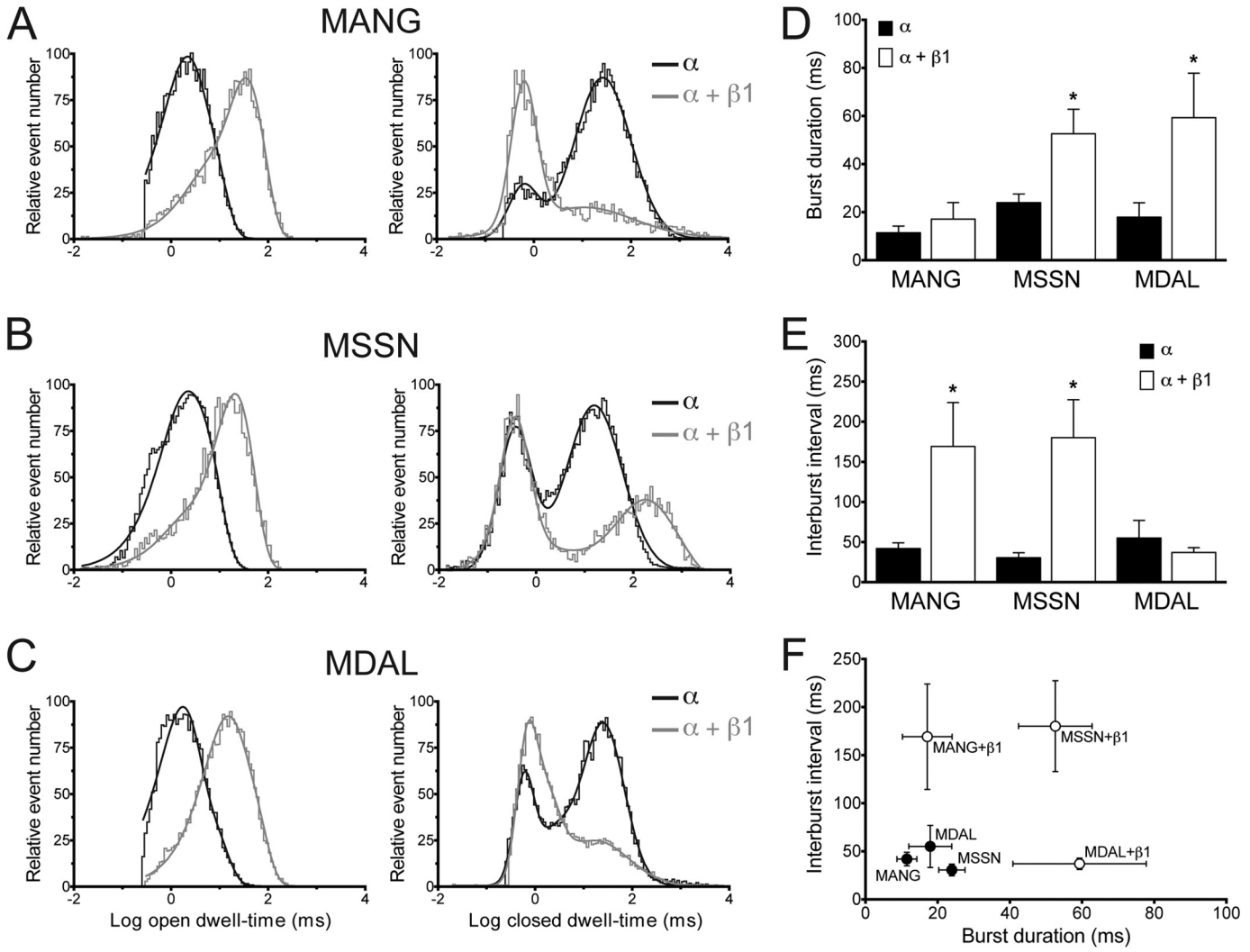

FIGURE 3. **BK_{Ca} channel kinetics of the N-terminal α-subunit isoforms are differentially regulated by the β1-subunit.** Open and closed dwell time distributions of single channels in HEK293T cells expressing MANG (*A*), MSSN (*B*), or MDAL (*C*) isoforms of the BK_{Ca} channel in the absence (*black lines*) or presence (*gray lines*) of β1-subunit ($n = 3–7$). Currents were evoked by holding the membrane potential at −20 mV for 3 min in the presence of 10 μM Ca^{2+} in the bath. Histograms were plotted in log-bin time scales and fitted with double exponential functions (*solid line*). Burst duration (*D*) and interburst (*E*) analyses of same recordings as in *A–C*. *, $p < 0.05$ compared with α-subunit alone. *F*, burst duration was plotted against interburst interval for each construct in the presence (*open symbols*) and absence (*closed symbols*) of β1-subunit.

TABLE 2

Effect of β1-subunit on the open and closed dwell-times of different BK_{Ca} α-subunit N-terminal isoforms

Time constants (τ_1 and τ_2) are expressed in milliseconds. P_1 and P_2 are relative distributions of data under curves used to fit the results shown in Fig. 3.

	Open, mean ± S.E.		Closed, mean ± S.E.	
Isoform	α	α + β1	α	α + β1
MANG				
τ_1	1.46 ± 0.09	2.82 ± 0.11[a]	1.2 ± 0.12	1.02 ± 0.07
P_1	0.49 ± 0.06	0.13 ± 0.01[a]	0.13 ± 0.01	0.69 ± 0.03[a]
τ_2	4.71 ± 0.08	34.15 ± 0.02[a]	35.55 ± 0.04	33.2 ± 0.23[a]
P_2	0.51 ± 0.06	0.87 ± 0.01[a]	0.87 ± 0.02	0.31 ± 0.03[a]
MSSN				
τ_1	1.06 ± 0.22	1.10 ± 0.18	0.61 ± 0.08	0.54 ± 0.05
P_1	0.32 ± 0.13	0.11 ± 0.01	0.36 ± 0.02	0.54 ± 0.02[a]
τ_2	3.60 ± 0.92	20.28 ± 0.02[a]	29.02 ± 0.04	40.34 ± 0.26[a]
P_2	0.78 ± 1.50	0.89 ± 0.01	0.64 ± 0.02	0.46 ± 0.03[a]
MDAL				
τ_1	1.45 ± 0.07	9.84 ± 0.05[a]	1.22 ± 0.09	1.54 ± 0.08[b]
P_1	0.68 ± 0.06	0.48 ± 0.03[a]	0.27 ± 0.02	0.66 ± 0.04[a]
τ_2	5.42 ± 0.15	32.13 ± 0.05[a]	35.35 ± 0.04	23.98 ± 0.28[a]
P_2	0.32 ± 0.06	0.52 ± 0.03[a]	0.73 ± 0.02	0.34 ± 0.05[a]

[a] $p < 0.01$ compared to α-subunit.
[b] $p < 0.05$ compared with α-subunit.

The diminished β1-induced shift in voltage-activation curves observed in the MANG isoform, and the lack of modulation in MSSN, were reflected in the modulation of channel kinetics. We observed that the β1-subunit modulated channel kinetics of all three BK_{Ca} α-subunit N-terminal isoforms, as evidenced by increases in open dwell times. We also observed that the closed dwell times of MANG and MDAL decreased, whereas the closed dwell times of MSSN increased; these data are consistent with other reports showing that the β1-subunit modifies the kinetics of the channel by stabilizing its open state (14, 18). The β1-subunit also increases the apparent Ca^{2+} sensitivity of the channel by increasing burst duration (20). Analysis of single-channel kinetics revealed that both MDAL and MSSN isoforms were maintained in the bursting state by β1, as indicated by an increased burst duration, but the burst duration of the MANG isoform was not affected by the β1-subunit. Furthermore, β1 increased the interval between bursts of MANG and MSSN isoforms but did not change the interval of the

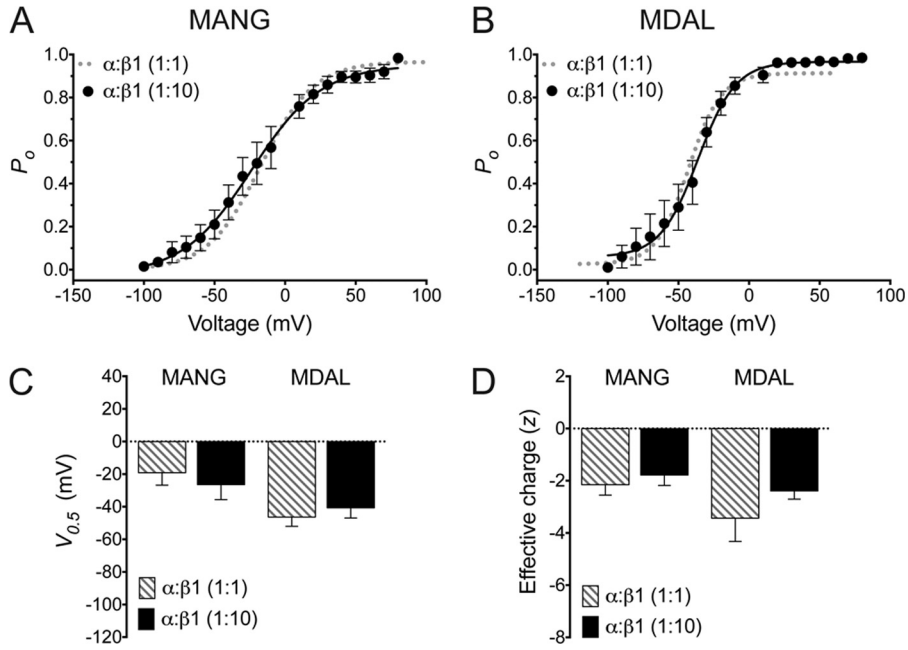

FIGURE 4. **Increasing α:β1 cDNA ratio does not affect the differential modulation by β1 of the MANG or MDAL isoforms.** Voltage dependence of BK_{Ca} activation of MANG (*A*) or MDAL (*B*) in the presence of the β1-subunit in HEK293T cells transfected at an α:β1 cDNA ratio of 1:1 (*dotted gray lines*; same data as shown in Fig. 2*D*, α+β1, 10 μM Ca^{2+} ($n = 6–7$)) or 1:10 (*closed circles* ($n = 8–10$)), expressed as open probability of the channel (P_o). The bath solution contained 10 μM Ca^{2+}. Half-maximal activation voltage ($V_{0.5}$, *C*) and effective charge (*z*, *D*) were obtained from voltage-activation curves for each isoform at 1:1 (*striped bars*) and 1:10 (*black bars*) α:β1 cDNA ratio.

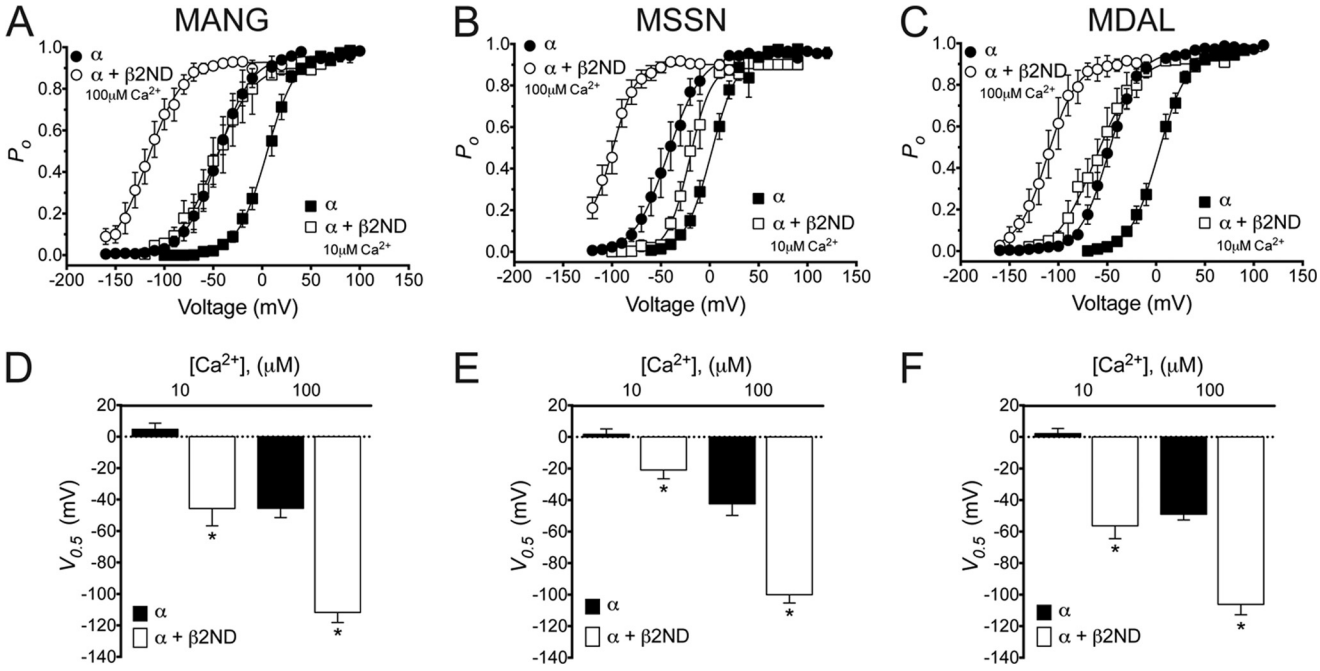

FIGURE 5. **The MANG, MSSN, and MDAL α-subunit isoforms are similarly modulated by the β2-subunit.** Shown is the voltage dependence of BK_{Ca} activation of MANG (*A*), MSSN (*B*), and MDAL (*C*) constructs in the presence (*open symbols*) or absence (*closed symbols*) of a β2-subunit construct with its N terminus deleted (*β2ND*), expressed as open probability of the channel (P_o) ($n = 6–12$). The bath solution contained 10 μM (*squares*) or 100 μM (*circles*) Ca^{2+}. Half-maximal activation voltage ($V_{0.5}$) obtained from voltage-activation curves for MANG (*D*), MSSN (*E*), or MDAL (*F*) isoforms for each Ca^{2+} concentration with (*open columns*) or without (*closed columns*) β2ND-subunit. *, $p < 0.01$ compared with α-subunit alone.

MDAL isoform. These observations (summarized in Table 3) support a model of allosteric modulation of BK_{Ca} channel kinetics by the β1-subunit in which the MANG-extended N-terminal sequence reduces the β1-dependent stabilization of the bursting state, but not the change in overall open and closed dwell times. In the MSSN isoform, the bursting state is stabilized by β1, but the time between bursts is longer, which might explain the net null effect of β1 on the voltage-activation of the MSSN isoform. Differential modifications by β1 in the burst duration and open/closed time constants of these channels could lead to changes in P_o and apparent Ca^{2+} sensitivity (20), altering membrane excitability. Thus, β1-dependent increase

TABLE 3
Summary of the β1-subunit effect on the different properties of BK_{Ca} channel α-subunit N-terminal isoforms

Isoform	Voltage dependence	Conductance	Open dwell time constants	Close dwell time constants	Burst duration	Interburst interval
MANG	Leftward shift	No change	Both increased	Decreased	No change	Increased
MSSN	No shift	No change	Only τ_2 increased	Increased	Increased	Increased
MDAL	Larger leftward shift	Decreased	Both increased	Decreased	Increased	No change

in burst duration of the MDAL isoform will result in an increase of the overall time the channel remains in the open state, a greater P_o, and a subsequent leftward shift in voltage activation.

The association between BK_{Ca} α- and its β1-subunit plays an important role in multiple tissues, most notably in regulating smooth muscle contractility (12, 27–31). For example, the β1-subunit controls arterial tone in resistance arteries (29) and contractility in the urinary bladder (31). Because the interaction between BK_{Ca} α and β1 seems to be important for human myometrial contractility (32, 33), and both subunits are abundantly expressed in this tissue (34), we explored the presence of the different N-terminal isoforms in smooth muscle tissue derived from human myometrium obtained from non-laboring pregnant women at term. However, detection of peptides corresponding to the various N termini by using mass spectrometry was unsuccessful (data not shown), likely because the low complexity of the sequence between amino acids Met-1 and Met-66 (polyglycine and polyserine stretches, Fig. 1) complicates the detection of these peptides by mass spectrometry. Further studies, such as N-terminal protein sequencing or development of specific antibodies targeted to the N-terminal region of the BK_{Ca} α-subunit, will be necessary to determine the endogenous expression of these isoforms.

Our data suggest that the additional N-terminal sequence in the MANG isoform might dampen the effects of the β1-subunit on channel activation by a specific allosteric mechanism rather than by hampering protein-protein interaction. The case of MSSN seems to be more complex; extending the N-terminal sequence by 41 amino acids completely abolished the modulation by β1 of the voltage-dependent activation observed with MDAL, but adding 24 more amino acids partially restored this modulation, as observed with the MANG isoform. The secondary structure of this N-terminal sequence is not clear because of its low complexity poly-glycine and poly-serine stretches (Fig. 1). Further studies aimed at dissecting the length of residues necessary to prevent and restore β1 modulation might elucidate the structural determinants of this effect.

Several studies have reported that the β1-subunit increases the Ca^{2+} and voltage sensitivity of recombinant BK_{Ca} channels (9–11, 13–15, 17, 18, 35–37). However, most reports used an α-subunit isoform starting at the third initiation site, the MDAL isoform (10, 14, 15, 17). Although three predicted initiation codons are encoded in the DNA sequence of the α-subunit (15, 16), neither the first nor second initiation sites (corresponding to MANG and MSSN isoforms, respectively) generate functional channels when expressed in *Xenopus laevis* oocytes (15). One study demonstrated that expression of functional BK_{Ca} channels might depend on the taxonomic class of the cell line used for heterologous expression. Erxleben *et al.* (9) described a BK_{Ca} channel in a rat pituitary cell line, starting at MSSN, whose kinetics are slower in the presence of β1, but lack the typical change in Ca^{2+} sensitivity at 1–10 μM Ca^{2+}. Interestingly, a truncated form starting at MDAL restores modulation by the β1-subunit. These authors propose that the polyserine stretch between Met-25 and Met-66 is important in blocking Ca^{2+} sensitivity modulation by β1. We found comparable results in that the human MSSN isoform lacked, and the MDAL isoform retained, voltage-dependent activation modulation by the β1-subunit. In our study, using a mammalian heterologous expression system, both MANG and MSSN N-terminal isoforms formed functional channels with intrinsic properties indistinguishable from those observed in the MDAL isoform. In addition, expression of the N-terminal isoforms in a mouse fibroblast cell line also generated functional channels, comparable to those expressed in HEK293T cells (data not shown). Thus, expression systems may account for the differences in BK_{Ca} channel N-terminal α-subunit function observed between our experimental conditions and those in other reports; however, the underlying mechanism for tissue specific expression of these N-terminal isoforms is still unclear.

The functional complexity of the N-terminal BK_{Ca} isoforms, and how their expression is regulated, has not been fully explored. Alternative translation initiation has been described to modulate the expression and function of N-terminal truncated forms of certain potassium channels: the voltage-gated potassium channel Kv3.3 (38) and the two P-domain potassium channels K_{2P}2.1 and K_{2P}10.1 (39, 40). Alternative translation initiation is a mechanism to regulate protein diversity whereby proteins with different N termini are produced from a single mRNA (41). Alternative translation initiation occurs during translation when the ribosome binds to an initiation codon (AUG) that is not the first cap-proximal in the mRNA coding region, thereby generating N-terminally truncated protein isoforms. Generally, AUG sequences are flanked by Kozak consensus sequences, which facilitate binding of the ribosome to the AUG sequence and thus translation initiation (42). The extent of this facilitation is determined by the relative strength of the ribosome binding given by the Kozak sequence; thus, some sequences will promote binding of the ribosome to a certain initiation site over others (43). In examining the mRNA sequence of the BK_{Ca} channel α-subunit, we found that the third initiation codon (Met-66) is flanked by a Kozak sequence stronger than those found in the first or second initiation codon (Met-1 and Met-25, respectively); this might lead to leaky scanning by ribosomes to initiate translation at MDAL. In our study, our N-terminal constructs contained an optimal Kozak consensus sequence (GACCACC) before their respective initiation codons to ensure their optimal heterologous expression. Nonetheless, it is possible that the expression of the MDAL isoform was facilitated by alternative translation initiation, at the expense of expression of MANG or MSSN isoforms; therefore,

additional studies are necessary to clarify the molecular mechanisms of this regulation.

Our data suggest that the relative expression of N-terminal isoforms can act as a novel mechanism of regulation of BK_{Ca} channel activity by the auxiliary β1-subunit. Thus, our results showing the differential activity of BK_{Ca} channel N-terminal isoforms might be relevant to several tissues and pathological processes in which BK_{Ca} channels, and their β1-subunits, are involved and could lead to development of specific therapeutic strategies to regulate channel activity.

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